REMARKS

Entry of the foregoing amendments, reconsideration and reexamination of the subject application, as amended pursuant to and consistent with 37 C.F.R. § 1.112, in light of the remarks that follow, is respectfully requested.

1. Status of the Claims

Claims 1-4 are pending. Claim 1 is independent.

Applicants have amended claims 1-4 to attend to some scientific references to degree Kelvin and grammatical issues. Additional amendments and support for the amendments are provided herein. However, it is believed that none of the claim amendments introduce prohibited subject matter. Amendments to the claims have been made without disclaimer of or prejudice to the claimed subject matter. Applicants reserve the right to file a continuation or divisional on any subject matter canceled by way of amendment to the claims.

2. Status of the Drawings

Applicants acknowledge that the Office has accepted the drawings filed on February 28, 2005.

3. Acknowledgement of Priority Under 35 U.S.C. § 119

Applicants note that the Office has received certified copies of the priority documents and have acknowledged the claim for priority under 35 U.S.C. § 371.

4. Acknowledgement of Information Disclosure Statements

Applicants note with appreciation the acknowledgement of the Information Disclosure Statements filed February 28, 2005, and May 4, 2005. Applicants noted that the Office did not acknowledge two references on the PTO-1449 filed February 28, 2005, because copies of the references were not enclosed. Applicants attach herewith copies of the references (Abstracts of the two Japanese patent applications and two journal articles) and enclose a new PTO-1449 form for the convenience of the Office. These references should have been forwarded by WIPO, thus Applicants request a credit of the fee if appropriate. Applicants request acknowledgement of the enclosed PTO-1449 with the next substantive response.

5. Claim Objections

Claim 1 is objected to because allegedly the temperature of the third step would necessarily fall beyond the range of the second step.

Applicants have amended claim 1 for the second and third steps according to the disclosure on pages 5-6 of the specification. The specification discloses temperature ranges for the various steps. On page 6, lines 3-6, the specification states that the temperature for the third step is more preferably 5-20°K lower than the diffusion onset temperature. The diffusion onset temperature of the second step falls in the range of 70~270°K. Accordingly, the range of 5-20°K lower than 70-270°K would result in a range of 50 to 265°K, in its broadest reading.

The fourth step requires that the temperature be higher than the diffusion onset temperature, but lower than 270°K. *See* page 6, line 14. The temperature can be between the diffusion onset temperature and 270°K. If below 270°K, there can be 30-50°K added to the temperature as long as the added temperature does not fall above 270°K. *Id.*, lines 12-17. Accordingly, and for purposes of clarity, Applicants have amended step four to read: "...raising the frozen mixture prepared in the third step to a temperature higher than the temperature of the third step but lower than 270°K ."

With the above amendments to the claims, Applicants submit that the objection is mooted and should be withdrawn.

6. Claim Rejections Under 35 U.S.C. § 112, First Paragraph

Claim 1 stands rejected under 35 U.S.C. § 112, first paragraph for allegedly lacking sufficient written description. The Office states that "[i]n the present instance, claim 1 recites the narrow recitation 80-270K, and the claims also recites 'and is higher than the temperature of the third step' which is the narrower statement of the range/limitation."

Applicants have amended step 3 to recite a lower range of temperatures. Applicants also have amended step 4 of claim 1 to recite a temperature that is higher than the temperature in step 3, but is lower than 270°K. Additionally, it should be noted that the temperature ranges provided in the second, third, and fourth steps of claim 1 are step specific. Thus, each temperature range is specific for each individual step in the method. The temperature ranges should not be construed as a range limitation to be read into the prior

step's temperature range. The steps instead are relational to each other for determining the appropriate temperature for each step. A skilled artisan reading the claims as well as the specification on pages 5-6 would have readily understood the meaning and utilization of the ranges. Accordingly, with the amendment, the rejection has been mooted and respectfully should be withdrawn.

7. **Claim Rejections Under 35 U.S.C. § 112, Second Paragraph**

Claim 1 stands rejected as indefinite for the recitation of "the temperature" in line 11. Without acquiescing as to the merits of rejection, Applicants have amended claim 1, such that "the temperature" is no longer recited, thereby mooting the rejection. Applicants accordingly respectfully request withdrawal of the rejection.

Claims 2-4 are rejected under 35 U.S.C. § 112, second paragraph, as indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Office's rejection on page 4 is restated below for the Office's convenience:

> Claim 2 initially recites the limitation "diffusion onset temperature", and the instant specification notes that the diffusion onset temperatures for at least two substrates are the same temperature in one instance, or differ by a range of 30K in another (Pg. 5, Lines 26-29). The instant specification also lists several more substrates and presumably any of the multitude of representative oxidoreductases can necessarily utilize other substrates not disclosed. Therefore, it would be impossible for the diffusion onset temperature and the claimed ranges to be the same in every embodiment.

Applicants disagree with the Office's characterization of the claims. Additionally, it is unclear why the Office requires that the diffusion onset temperature and the claimed ranges to be the same in every embodiment. The temperatures do not need to be the same for every embodiment. However, the diffusion onset temperature would be the same for each individual substrate proceeding to diffuse through the mixture; however, the diffusion onset temperatures would vary as between substrates, as would have been understood the skilled artisan. The diffusion onset temperature may only vary between substrates being used. Nevertheless, for clarity, Applicants have amended claim 2 to further set forth diffusion onset temperatures and any ranges defined from the value of the diffusion onset temperature. In

view of the amendments to claims 2-4, it would be clear to the skilled artisan what the steps of the claimed method encompasses, placing the artisan in possession of the invention. Accordingly, Applicants respectfully request withdrawal of the indefiniteness rejection as to claims 2-4.

8. Claim Rejections Under 35 U.S.C. § 103

Claims 1-4 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Geren et al., ("Design of a ruthenium-cytochrome c derivative to measure electron transfer to the initial acceptor in cytochrome c oxidase," *J. Biol. Chem.* 270(6): 2466-2472, 1995) (hereinafter "Geren") in view of Schlichting et al. ("The catalytic pathway of cytochrome P450CAM at atomic resolution," *Science* 287: 1615-1622, 2000) (hereinafter "Schlichting").

Applicants traverse the rejection and assert that a *prima facie* argument of obviousness has not been adduced for any of the four claims. A finding of obviousness under 35 U.S.C. § 103 requires a determination of the scope and content of the prior art, the differences between the invention and the prior art, the level of ordinary skill in the art, and whether the differences are such that the claimed subject matter **as a whole** would have been obvious to one of ordinary skill in the art at the time the invention was made. *Graham v. John Deere Co.*, 383 U.S. 1 (1966). The prior art must be viewed for what it or the combination teaches as a whole ("The test for an implicit showing is what the combined teachings, knowledge of one of ordinary skill in the art, and the nature of the problem to be solved as a whole would have suggested to those of ordinary skill in the art.") *In re Kotzab,* 217 F.3d 1365, 1370, 55 U.S.P.Q.2d 1313, 1317 (Fed. Cir. 2000); *see also* M.P.E.P. § 2143.01. Secondary considerations, such as unexpected results, failure of others, etc., which are indicia of non-obviousness must be taken into account, if present. *Fromson v. Advance Offset Plate, Inc.*, 755 F.2d 894, 904 (Fed. Cir. 1988). Once the scope and content of the prior art is determined, the relevant inquiry is whether the prior art suggests the invention, and whether one of ordinary skill in the art would have had a reasonable expectation that the claimed invention would be successful. *In re Vaeck*, 20 U.S.P.Q.2d 1438, 1442 (Fed. Cir. 1991). Both the suggestion of the claimed invention ***and*** the expectation of success must be in the prior art, not in the disclosure of the claimed invention. *In re Dow Chemical Co.*, 5 U.S.P.Q.2d 1529, 1531-1532 (Fed. Cir. 1988) (emphasis added). If an explicit suggestion or teaching is missing from a reference, it cannot be supplied by an inherent feature to support an obviousness rejection. *In re Sernaker*, 217 U.S.P.Q. 1, 6 (Fed. Cir. 1983). There must be

some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify or combine reference teachings. *See* M.P.E.P. § 2145.X.C. (Lack of Suggestion to Combine Reference). Additionally, "obvious to try" is not the legal standard for adducing obviousness. The Office is suggesting varying all the parameters and to try each of the numerous possible choices until a successful result is achieved. Just because someone could have done this, does not rise to rendering the claimed invention obvious.

Geren is cited for allegedly disclosing "[d]issolving the oxidoreductase cytochrome-c oxidase, the photoinduced reducing agent Ruthenium (bipyridine), the substrate heme c and the amine-type donor aniline in buffer...and irradiating the mixture with a light in a wavelength region including the absorbing wavelength of the photoinduced reducing agent in order to induce a reaction." Office Action, page 5.

Applicants assert that the primary reference, Geren, is directed to solving a problem using a different method than the problem and method used by Schlichting or taught in the instant application, such that there is no explicit or implicit motivation to combine. Geren is directed to laser excitation of a complex in a low ionic strength buffer that is not frozen and the impact of increasing the ionic strength of the buffer. The reduction rate in Geren is very low, *i.e.*, about 0.6% (0.11/1.8) as calculated from page 2469, line 8 after Table 1. The reduction rate using the claimed method can reach toward 100%. In Geren, the Ruthenium (Ru) complex is surrounded by cyt c and amine (aniline), and thus cannot give an electron to the Ru complex, which hampers electron transfer. The complex of the claimed method has the Ru closely bonded to cyt c, which prevents the Ru complex from easily contacting the amine (aniline). This allows the Ru-39-cyt c (electron donor) to transfer an electron to the enzyme, and the Ru complex becomes a strong oxidizing agent. Geren is therefore distinguishable from the claimed method.

Geren **does not** teach mixing the compounds in water but only in a buffer at a certain pH. Applicants do not claim using a buffer or a certain pH. Geren appears to perform the reactions at 25°C (*i.e.*, 298.15°K) and not at 70-270°K (*i.e.*, -203.15°C to -3.15°C). Nor does Geren appear to discuss the specific steps of lowering and raising the temperature of the frozen mixture. Indeed, the reactions of Geren do not appear to be frozen at all ("Solutions containing...were placed in semimicro glass cuvettes. The excitation pulse was provided...." Page 2467, right col.). As acknowledged by the Office, Geren does not teach any

temperature regulation with respect to the diffusion onset temperature, let alone variation of the temperature with regard to that temperature or range of temperatures.

Schlichting is asserted as a secondary reference to cure the deficiencies of Geren. Schlichting alone and in combination does not suggest the claimed method.

The teachings of Schlichting, and especially that of Table 1 (page 1616) were asserted by the Office to cure the deficiencies of Geren (Schlichting was a secondary reference). Schlichting was cited for teaching a method comprising dissolving the oxidoreductase, a substrate and electron donors. Schlichting also uses a reducing agent, which requires adding oxygen. The claimed method does not use a reducing agent, but rather a photoinduced reducing agent that does not require additional oxygen. The mixture was then frozen at 88°K, irradiated with X-rays at 96°K, raising the temperature to 100°K and then to 293°K. Office Action, para. bridging pages 6-7. X-rays cause damage to the crystal protein and their use is not suitable for studying such delicate natural phenomena. The claimed method does not use X-rays.

Schlichting **does not** teach adding an amine type electron donor. Schlichting **does not** teach the use of water, as claimed in claims 1-4. Schlichting teaches raising the temperature to 293°K (*i.e.*, 19.8°C). Raising the temperature above the claimed amount of 270°K to 293°K would allow the frozen mixture to thaw. Applicants do not teach thawing the mixture. Thawing will also damage the enzyme and therefore is not a desired step. Schlichting additionally teaches the following for the description of Table 1 on page 1616:

> ...we used polyethylene glycol (PEG)- grown monoclinic (and orthorhombic) (24) crystals for all experiments performed at cryogenic temperatures. These crystals (0.03 mm by 0.06 mm by 0.3 mm) were grown in sitting drops by mixing 3 to 5 µl of P450 (30 mg/ml) with equal volumes of the reservoir solution [50 mM tris-HCl (pH 7.4), 250 mM KCl, 100 mM DTE, 1 mM camphor, and 27 to 30% PEG 4000]. Time-resolved spectroscopic analysis of microcrystalline slurries was used to obtain the conditions and time windows for generating the ternary P450.camO_2 complex (see Fig. 3) before it decays because of autooxidation (9). For reduction, crystals were soaked at 2°C in nitrogenated mother liquor containing 50 mM dithionite and 40 mM NaOH until a color change occurred (30 min). Subsequently, the crystals were rinsed in nitrogenated mother liquor for 1 min. Oxygen complexes were made by exposing reduced crystals mounted in a loop with mother liquor containing 20% glycerol to 120-bar oxygen atmosphere for 3 min at 2°C. Then the pressure was released over a period of 30 to 60 s, and the crystals were flash cooled in liquid nitrogen. As described in the text and in this table, three data sets were collected

> of P450.camO_2 crystals kept at cryogenic temperature with an Oxford cryostream. After collection of the first data set (s1) with sort-wavelength X-rays, the crystals were exposed to long-wavelength x-rays for about 3 hours, and then the second data set (s2) was collected. The third data set (s3) was collected after briefly thawing the crystals.

Applicants' method teaches that the temperature remain at or below 270°K. Temperatures 5-10 degrees in excess of that amount would induce thawing. Thawing is required apparently in order to obtain the third set of data reported in Schlichting. Schlichting also appears to require observation of a color change, which is not required by the instant method. Schlichting is directed to taking data points at different temperature and time points. The reference does not teach keeping the mixture temperature below 270°K, the specific steps of temperature control, and the ranges of temperature control discussed in the subject application. The assertion on page 8 of the Office Action that thawing the sample is necessary for the reaction to proceed does not obviate the fact that Applicants do not teach thawing. Applicants instead require that the reaction mixture remain frozen at all steps in the claimed method. The fact that some of the temperatures used by Schlichting happened to be below the diffusion onset temperature of the substrate is insufficient when viewed in what Schlichting teaches as a whole to overcome all the other differences in the reference. These numerous differences as taught by Schlichting are distinguishable from the claimed invention. Moreover, the differences taught by Schlichting negate a motivation to combine the references, let alone overcome the other deficiencies of the Geren reference.

Thus, the references when viewed alone do not teach the claimed invention. Additionally, with all the variables as between the two references, there is no motivation to combine the references to achieve the combined claimed set of steps. The references accordingly cannot provide any expectation of success that the asserted combination of steps would be successful. Accordingly, the Office has not adduced a *prima facie* case of obviousness, and the rejection should respectfully be withdrawn.

CONCLUSION

In conclusion, this amendment and reply is believed to be a full response to the outstanding Office Action. Should any issues remain outstanding or if there are any questions concerning this paper, or the application in general, the Examiner is invited to telephone the undersigned representative at the Examiner's earliest convenience.

EXCEPT for issue fees payable under 37 C.F.R. § 1.18, the Commissioner is hereby authorized by this paper to charge any additional fees during the entire pendency of this application including fees due under 37 C.F.R. §§ 1.16 and 1.17 which may be required, including any required extension of time fees, or credit any overpayment to Deposit Account No. 50-0573. This paragraph is intended to be a **CONSTRUCTIVE PETITION FOR EXTENSION OF TIME** in accordance with 37 C.F.R. § 1.136(a)(3).

Respectfully submitted,

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